IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Tullis, *et al.*

Serial No.: 09/917,138

Confirmation No: 4876

Filed: July 26, 2001

For: *ENZYMATIC LABELING AND DETECTION OF DNA HYBRIDIZATION PROBES*

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Assistant Commissioner for Patents
Washington, D.C. 20231, on this date.

02/08/02
Date

Lynn Morkunas

PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph on page 1, line 18, to line 22, with the following paragraph.

DNA probes and primers have found a variety of commercial and research applications in DNA hybridization diagnostics including DNA and RNA target amplification technologies (PCR, LCR and NASBA); signal amplification technologies such as branched DNA probes, dendrimers and the like; and direct DNA probes for less sensitive detection.

Please amend the paragraph on page 3, line 22, to page 4 line 13, with the following paragraph:

Another system that has been applied to genotyping is the Taqman system (Perkin Elmer, Foster City, CA). In the Taqman paradigm (see, *e.g.*, Holland *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:7276-7280), fluorescent energy-transfer probes known as Taqman probes or Molecular Beacons have been employed in a homogeneous format to detect amplification products. A Taqman probe includes a fluorescent donor and fluorescent quencher typically attached to the 3′ and 5′ ends of a sequence specific oligonucleotide (SSO). In

a Molecular Beacon, the quencher is a non-fluorescent chromophore, such as, but are not limited to, DABCYL (4-(4-dimethylaminophenyl)azobenzoic acid; see, *e.g.*, Kostrikis *et al.* (1996) *Science 279*:1228-1229) and EDANS (5-((2-amino-ethyl)amino)-naphthalene-1-sulfonic acid), which is fluorescent group quenched by the DABCYL group. During amplification, the exonuclease activity of Taq polymerase cleaves the probe between the quencher and the fluor, causing a directly [observably]observable increase in fluorescence of from 3-20 fold. The Taqman system combines the amplification and detection in a closed system reducing the risk of contamination and allowing multiplex detection. There are drawbacks to this system. Taqman probes vary substantially in quenching efficiency and are difficult to synthesize and purify. As a result, the system tends to be less robust than typical clinical systems and cannot use highly modified DNA probes that are resistant to nucleases. Moreover, Taqman probes and the associated instrumentation to detect fluorescence changes can be quite expensive.

Please amend the paragraph on page 6, line 29, to page 7 line 11, with the following paragraph.

As used herein, a non-template dependent chain extending enzyme refers to template independent polymerases capable of adding polynucleotide tails to the termini of DNA or RNA molecules. Chain [expending]extending enzymes include, but are not limited to, telomerases such as terminal transferases, that are capable of producing extended polynucleotide tails. Telomerases extend the 3′ termini of chromosomes thereby stabilizing chromosomal structure. Assays to identify telomerases are known (see, *e.g.*, U.S. Patent Nos 5,489,508; 5,645,986 and 5,648,215). Generally telomerase activity is measured by primer chain elongation under conditions that minimize interference from other genomic sequences. For example, U.S. Patent No. 5,629,154 describes telomerase activity assays. In these assays, telomerase activity in a

sample is measured using a two reaction protocol involving telomerase substrate and primer extension steps.

Please amend the paragraph on page 14, line 7, to line 19, with the following paragraph.

The products of the chain extension reaction can then be detected by suitable methods known to those of skill in the art. [Suche]<u>such</u> methods include, but are not limited to:

1) Direct luminescent detection via incorporated fluorescence or chemiluminescent nucleoside triphosphates.
2) Indirect fluorescence or chemiluminescence mediated by antibodies, streptavidin or other lectins or aptamers
3) Enzymatic reporter groups attached to antibodies, streptavidin or other lectins or aptamers
4) Up [convering]<u>converting</u> phosphors or fluorescent beads attached to oligomers.

Hence, suitable labels include any detectable label that can be incorporated into an extended chain.

IN THE CLAIMS

Please amend Claims 1 and 10 as follows:

1. A method, comprising:

a) treating nucleic acid molecules or modified nucleic acids in a sample with a reagent or reagents that render the nucleic acid chains unextendable by a non-template-dependent enzyme; and

b) hybridizing the treated molecules with a nucleic acid probe that includes an extendable terminus, under conditions whereby hybrids form; and

c) treating any hybrids formed with [an]<u>a</u> non-template dependent chain elongating enzyme and substrates therefor, whereby any hybridized probe is extended.

10. The method of claim [4]9, [where in]wherein the telomerase is terminal deoxynucleotidyl transferase.

* * *

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: _____
Stephanie Seidman
Registration No. 33,779

Attorney Docket No. 24730-2207B
Address all correspondence to:
Stephanie Seidman
HELLER EHRMAN WHITE & McAULIFFE LLP
4350 La Jolla Village Dr., 6th Floor
San Diego, California 92122-1246
Telephone: 858 450-8400
Facsimile: 858 587-5360
email:sseidman@HEWM.com